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(54) **An instrument and a method for measuring the activity of an enzyme or the activity of an enzymatic reaction-inhibiting substance.**

(57) An instrument for measuring the activity of an enzyme or the activity of an enzymatic reaction-inhibiting substance, which comprises a thin tube section charged with a substrate gel for an enzymatic reaction and provided on one end thereof with a receptacle section for a sample liquid to be measured.

Fig. 1

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The present invention relates to an instrument and method for simply measuring the activity of an enzyme or the activity of an enzymatic reaction-inhibiting substance. The present invention is particularly useful for the measurement of activity of enzymes or the activity of enzymatic activity-inhibiting substances in living body samples in the field of clinical inspection.

For the measurement of enzymatic activities, determination of a decomposition substance of a substrate is widely carried out, which has been obtained by reacting the substrate with the enzyme. For example, a method for measuring absorbance of a particular decomposition substance from a substrate, a method for measuring a radioactive isotope in decomposition substances from a substrate labelled with the isotope, a method for the assay of a particular functional group in decomposition substances from a substrate, etc. have heretofore been known as the measurement of enzymatic activities.

The principle of measurement of enzymatic activity in the present invention resides in bringing a sample liquid containing an enzyme into contact with a substrate gel for use in enzymatic reaction in a thin tube to cause the contemplated enzymatic reaction, measuring the volume of the substrate gel a part of which is dissolved as a result of the enzymatic reaction, and making assay of enzymatic activity based on the result of measurement.

The principle of measurement of the activity of an enzymatic reaction-inhibiting substance in the present invention resides in bringing a sample liquid containing an unknown amount of the enzymatic activity-inhibiting substance to be measured and a given amount of an enzyme into contact with a substrate gel for use in the enzymatic reaction in a thin tube thereby causing the enzymatic reaction, measuring the volume of the substrate gel a part of which is dissolved as a result of the enzymatic reaction, and making assay of the activity based on the result of measurement. In this case, the activity of the enzymatic activity-inhibiting substance is represented by a value obtained by deducing the actually measured enzymatic activity from the enzymatic activity which would be obtained from the given amount of the enzyme.

As the enzymatic reaction is carried out in a thin tube, the measurement of a substrate gel can easily be made in the present invention.

Any of the substrate gels can be used for the present invention so far as they can react with a particular enzyme selected and can be dissolved in a liquid used. Illustrative of such substrate gel are, for example, conventionally known gelled proteins such as collagen and fibrin. The enzyme as an object of measurement of its activity is selected in relation to the substrate gel. For example, a protease is an object enzyme for gelled proteins. More particularly, collagenase is selected for a substrate gel comprising collagen while plasmin for a substrate gel comprising fibrin.

An instrument used for practice of the present invention comprises a thin tube section for being charged with a substrate gel for use in the intended enzymatic reaction and a receptacle section for a sample liquid to be measured which is fitted to one end of the thin tube section. Fig. 1 is a perspective view showing one example of the instrument wherein the reference numeral 1 is a thin tube section charged with a substrate gel G and is provided on the upper end thereof with a receptacle section 2 in the form of a thick tube for a sample liquid to be measured, which is shaped integrally with the thin tube section, 3 is an attachment for adapting itself to a pipetter formed on the upper part of the receptable section 2, and 4 is a sealed end portion at the lower end of the thin tube section. The attachment 3 may be omitted.

In order to charge the substrate gel G into the thin tube section 1, the attachment 2 is mounted to the pipetter through which the liquid substrate is sucked into the thin tube section. The lower end of the thin tube section 1 is then sealed and the liquid substrate is gelled in the thin tube section. A proper method can be used for the gelation of the liquid substrate according to the sort of substrate.

Example 1

Basement membrane matrigel (trade name) containing Collagen Type IV as a predominant ingredient (manufactured by Collaborative Research, U.S.A., Catalog. No. 40234) was maintained at normal temperature to convert it into a solution and was taken up in a thin tube section 1 (inner diameter: 0.5 mm, length: 2,5 cm) having the structure as shown in Fig. 1 (manufactured by Quality, U.S.A., Catalog. No. 010). The lower end of the thin tube section 1 was heat-sealed by fusion. The liquefied collagen was solidified (gelled) by allowing it to stand at room temperature for about one hour. The volume of the liquid necessary to fill the thin tube section was about 4 μ l.

Dispase (trade name) manufactured by Godo Shusei KK which is a protease originated from Bacillus polymyxa and having the activity of collagenase type IV was diluted to various concentrations. In the receptacle section 2 was placed 50 μ l of a diluted Disperse solution as a sample liquid to be measured and the substrate G was overlaid with the sample liquid which was then allowed to stand for 72 hours at 37 °C to effect reaction. The sample liquid and the dissolved substrate gel were separated and the length of

the remaining substrate gel was measured. A result of the measurement is shown in Table 1. A similar experiment was carried out in the same manner using trypsin, a result of which is shown in Table 2.

Table 1

enzyme (μg/ml) concentration	the length of the remaining gel (mm)
62.5	4.60
31.25	3.60
15.6	2.90
7.8	1.50
3.8	1.10
1.9	0.85
1	0.40

Table. 2

enzyme concentration(μg/ml) (μg/ml)	the length of remaining gel (mm)	
	first	second
2000	22.60	22.10
1000	21.80	20.70
500	18.80	20.30
250	15.60	16.20
125	14.40	14.70
62.5	11.20	11.10
31.25	5.50	6.20
15.6	0.80	1.00
7.8	0.10	0.60

Claims

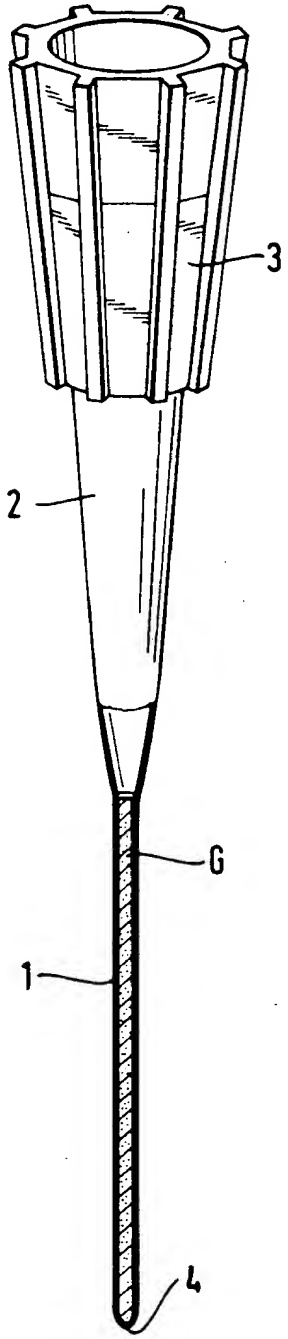
1. An instrument for measuring the activity of an enzyme or the activity of an enzymatic reaction-inhibiting substance, which comprises a thin tube section charged with a substrate gel for an enzymatic reaction and provided on one end thereof with a receptacle section for a sample liquid to be measured.

2. An instrument according to claim 1, wherein the thin tube section has a constant inner diameter.

3. A method for measuring the activity of an enzyme, which comprises charging a thin tube with a substrate gel for an enzymatic reaction, bringing a sample liquid to be measured into contact with the substrate gel in the thin tube thereby causing the enzymatic reaction, and measuring the volume of the substrate gel partially dissolved after the reaction.

4. A method for measuring the activity of an enzymatic reaction-inhibiting substance, which comprises charging a thin tube with a substrate gel for an enzymatic reaction, bringing a sample liquid to be measured which contains an enzymatic reaction-inhibiting substance and a given amount of an enzyme into contact with the substrate gel in the thin tube thereby causing the enzymatic reaction and measuring the volume of the substrate gel partially dissolved after the reaction.

Fig. 1



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EUROPEAN SEARCH REPORT

Application Number

EP 91 12 1925

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	APPLIED MICROBIOLOGY, vol. 14, no. 6, November 1966, USA, pages 892-898; G.S. RAUTELA et al: "Simple Cultural Test for Relative Cellulolytic Activity of Fungi" * pages 892-894 *	1-4	C 12 Q 1/00 C 12 M 1/00 // C 12 Q 1/37 C 12 Q 1/34
A	EP-A-0 319 334 (BLOCK DRUG CORP.) * abstract *	1-4	
A	US-A-4 506 010 (N. GOODMAN et al.) * abstract *	1-4	
A	EP-A-0 329 190 (SHOWA DENKO KABUSHIKI KAISHA)		

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 12 Q
C 12 M

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	08-04-1992	HITCHEN C.E.

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